Title of Trial: A randomized, controlled, multicenter, phase I/II study of cetuximab plus irinotecan investigating the pharmacodynamics and -genomics as well as the safety and efficacy of a dose escalation schedule of cetuximab compared with the standard fixed dosing regimen in patients with irinotecan-resistant EGFR expressing metastatic colorectal cancer

Investigational Product: Cetuximab plus irinotecan

Trial No.: EMR 62202 – 502 (EVEREST= **E**valuation of **V**arious brbitux **RE**gimens by means of **S**kin and **T**umor biopsies).

Study Centers: This study was conducted in 14 centers in 7 countries; 5 in Belgium, 2 in Netherlands, 2 in Germany, 2 in Sweden, 1 in France, 1 in Italy and 1 in Switzerland.

Trial Initiation Date: 05 May 2004

Trial Completion Date: 31 July 2006 (Clinical cut-off date)

Development Phase: Phase 1/2

Publications (references):

Van Cutsem E, Humblet Y, Gelderblom H, et al. ASCO Gastrointestinal Cancers Symposium 2007: abstract 237.

Van Cutsem E, Humblet Y, Gelderblom H, et al. Ann Oncol 2006; 17 (supplement 9): abstract LBA4.

Tejpar S, Peeters M, Humblet Y, et al. J Clin Oncol 2006 ASCO Annual Meeting Proceedings Part I; 24 (18S, June 20 Supplement): abstract 3554.

Study Objectives:

Primary Objective:

- To compare in skin biopsies the effects of a cetuximab dose escalation regimen on epidermal growth factor receptor (EGFR) and downstream signaling pathway markers with those of the standard cetuximab regimen.

Secondary Objectives:

- To compare percentages of subjects experiencing grade ≥2 skin toxicity in subjects randomized to receive cetuximab at the standard dosing regimen (dose group A) and in subjects randomized to receive an escalating dosing regimen (dose group B) using US National Cancer Institute's – Common Toxicity Criteria (NCI-CTC), version 2.0.

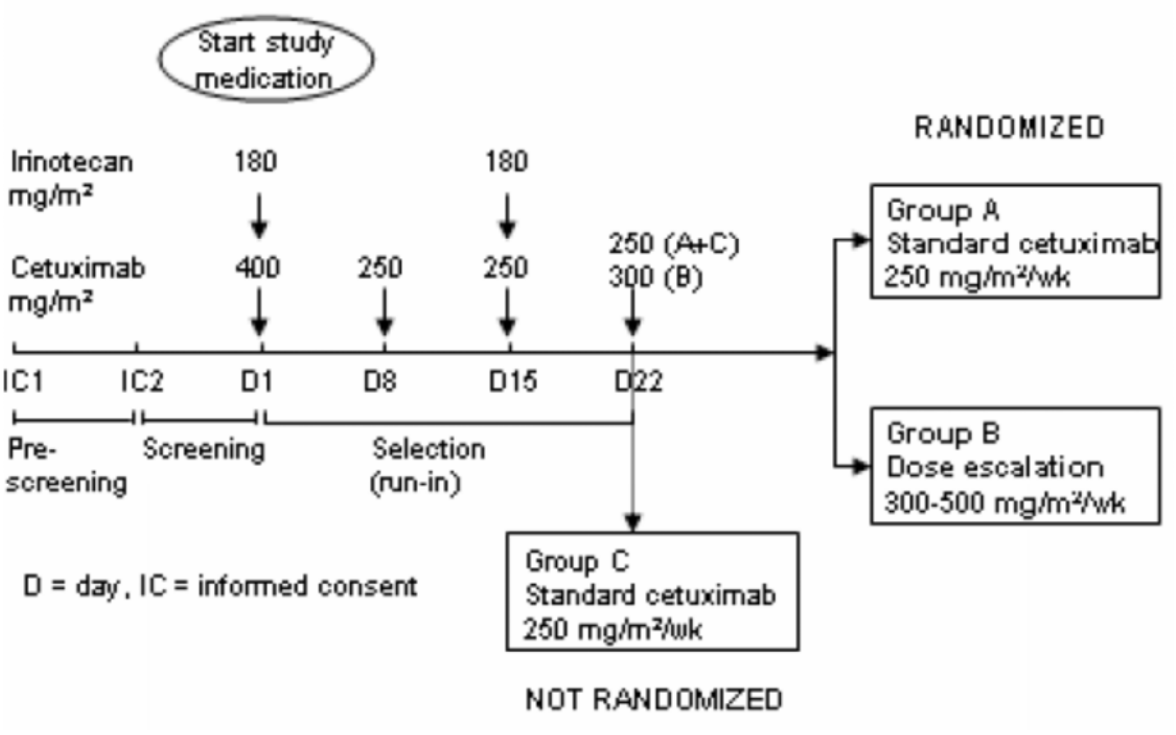
- To compare groups A and B with respect to efficacy and toxicity, with efficacy assessed as overall confirmed response rate, time to progression, duration of response, overall

survival time and disease control rate; and toxicity (safety) assessed as adverse events (AEs) (especially skin toxicity) and tolerability of the cetuximab regimens.

- To investigate effects of higher cetuximab doses on EGFR and other downstream signaling pathway markers in tumor samples.
- To investigate the relationship between efficacy, skin toxicity and alterations in molecular profiles to identify biomarker profiles that correlate with response or resistance.
- To investigate differences in pharmacodynamic markers between baseline and on-treatment samples.
- To evaluate pharmacokinetics (PK).

Methodology:

This was an open, randomized (1:1), and controlled, multicenter study of cetuximab in combination with irinotecan (180 mg /m² every 2 weeks) in subjects with metastatic, EGFR-expressing colorectal cancer (CRC) who had progressed on a defined irinotecan-based regimen as most recent pre-study treatment. The study design is summarized below.



Subjects with EGFR-expressing tumors entered screening for inclusion and exclusion criteria within 21 days of the planned start of study treatment (day 1). All subjects received the standard cetuximab regimen for 3 weeks. At day 22, subjects were randomized to the standard

cetuximab regimen (group A) or the dose-escalation regimen (group B). Subjects were only eligible for randomization if they had not experienced a skin reaction >grade 1, any other cetuximab-related toxicity >grade 2 or intolerance to irinotecan requiring discontinuation since the start of study treatment; they also had to provide a skin and tumor biopsy between days 18 and 22.

An end of study visit was performed 42 days after the last cetuximab dose for assessment of human antichimeric antibodies (HACAs) and follow-up for ongoing AEs. Follow-up for survival was performed every 12 weeks after the end of study visit.

Number of Subjects (Planned and Analyzed):

275 prescreened, 219 with EGFR-expressing tumors, 166 treated (group A 45, group B 44, group C 77). See flowchart in 'Disposition of subjects' below.

Study Population	Number of Subjects Analyzed			
	Group A	Group B	Group C	Total
Safety	45	44	77	166
Intention to treat (ITT)	45	44	68	157
Skin toxicity	45	44	44	133
Target immunohistochemistry (IHC)	18	26	-	44
Pharmacokinetic	34	27	42	103

Diagnosis and Main Criteria for Inclusion/Exclusion:

Adult subjects with metastatic, EGFR-expressing, histologically confirmed adenocarcinoma of the colon or rectum that was not suitable for curative-intent treatment. Subjects had to show documented progression of disease (time between documentation of progression and start of study treatment was not to exceed 90 days). They also had to have received irinotecan (180 or 210 g/m² every 2 weeks or 350 g/m² every 3 weeks as monotherapy or in combination with other agents) for at least 6 weeks as most recent prestudy chemotherapy treatment.

Study Treatment:

Test product: Cetuximab 2 mg/mL solution in 50 mL vials (i.e. 100 mg cetuximab/vial).

Dose: Subjects in groups A and C received the standard cetuximab regimen: initial dose 400 mg/m² followed by weekly doses of 250 mg/m². Subjects in group B received this regimen until day 21; from day 22 the cetuximab dose was escalated by 50 mg/m² every 2 weeks provided subjects had not experienced skin toxicity >grade 2, any other cetuximab-related toxicity >grade 2, or a partial or complete tumor response. Maximum cetuximab dose 500 mg/m².

Mode of administration: The mode of administration was intravenous fusion. Cetuximab was administered in combination with irinotecan (180 mg/m² every 2 weeks, intravenous infusion) in all treatment groups.

Duration of treatment: Until progression of disease, unacceptable toxicity, or withdrawal of consent.

Criteria for Evaluation:

Biomarkers:

Pharmacodynamics: Immunohistochemistry (IHC) analysis of selected markers associated with the EGFR signaling pathway and cell cycle in non-lesional skin biopsies (primary biomarker endpoints) and tumor biopsies.

Pharmacogenomics: Gene (ribonucleic acid) expression profiling of tumor biopsies, proteomic analysis of 97 candidate proteins in plasma.

Efficacy: Best overall tumor response assessed every 6 weeks by investigator (modified WHO criteria), progression-free survival (PFS) time and rates, survival time and rates.

Safety: AEs, skin toxicity, physical examination, vital signs, electrocardiogram, cardiac ejection fraction, clinical laboratory evaluations, HACAs.

PK: Serum concentration of cetuximab and PK parameters at weekly intervals (trough concentrations) and on intensive sampling (day 29 for groups A and C; 8th day of dose escalation regimen for group B [5 subjects per dose level]).

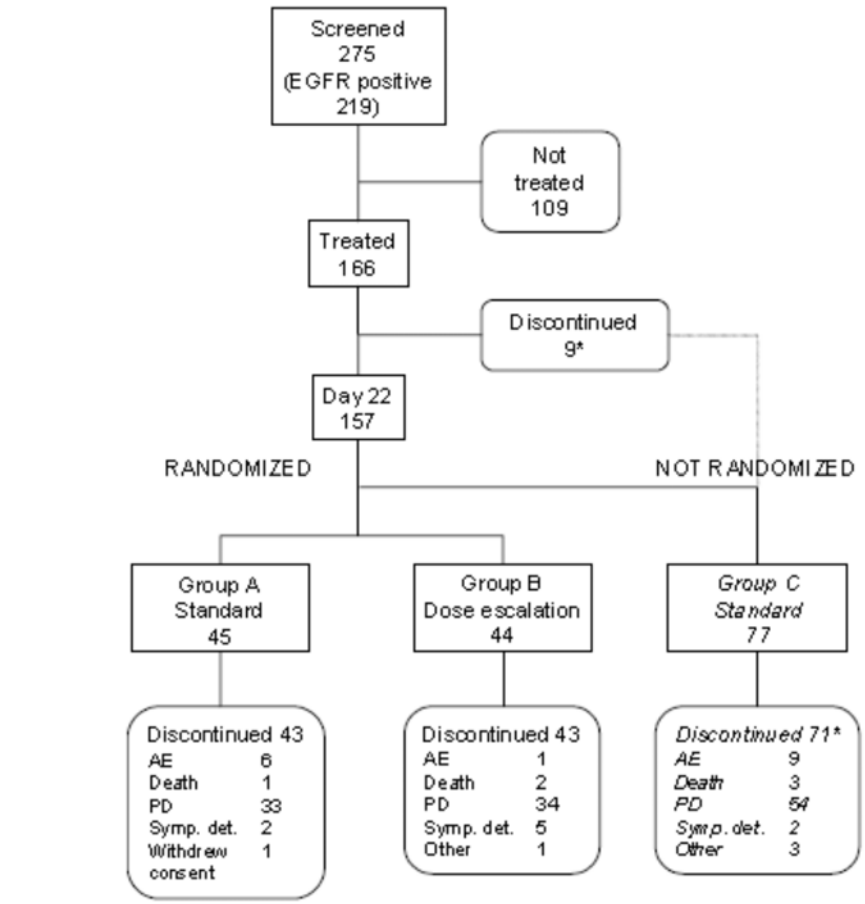
Statistical Methods:

The statistical analysis was conducted on all case report form and survival data collected up until the cut-off date (31 July 2006). Analyses of subject disposition, demographic and baseline characteristics, efficacy (tumor response and survival), and safety were performed on the ITT, safety and skin toxicity populations using descriptive statistics. Efficacy analyses were supportive and analyzed in a non-confirmatory sense.

All biomarker analyses were purely explorative. General biomarker analyses were performed on the ITT population. The primary biomarker endpoints (pharmacodynamics markers in skin determined by IHC) were analyzed in the target IHC population.

Results

Subject Disposition: The subject disposition results are presented below:



* The 9 subjects who discontinued before day 22 are included in the 71 discontinuations in group C.

Demographics and Baseline Characteristics: of the 2 randomized treatment groups A and B were comparable and typical of a previously treated study population with metastatic CRC who had failed irinotecan-based therapy. Mean ages (S.D.) were 58 (9.8) and 59 (9.8) years, respectively; 26 (58%) and 27 (61%) of subjects were men. All subjects had a baseline Karnofsky performance status (KPS) above 80.

Biomarker Results: The numbers of subjects with samples for biomarker analyses were too low to allow conclusive statements to be made. All analyses were of an exploratory nature.

IHC analysis of non-lesional skin biopsies did not reveal any clinically relevant effects of dose escalation of cetuximab on the investigated

Pharmacodynamics markers (EGFR, pEGFR, nuclear pERK, cytoplasmic pERK, pSTAT3, Ki67 and p27) related to EGFR signal transduction and the cell cycle. In general, none of these biomarkers showed a robust dynamic on-treatment change within either the different dose groups or the response groups. Likewise, IHC analysis of tumor biopsies did not reveal any relevant effects of higher cetuximab doses on EGFR, pEGFR, HER2, pHER2, nuclear pERK, cytoplasmic pERK, pAkt, pSTAT3, Ki67, p27 or CC3 in tumor samples.

Overall, no major biomarker baseline profiles could be detected in IHC analysis of skin or tumor biopsies or proteomic analysis of plasma samples that showed a robust association with efficacy or skin toxicity. In the IHC analysis of tumor biopsies, moderately lower levels of p27 and higher levels of nuclear pERK at baseline were associated with response. Subjects with a partial response had a trend for lower baseline plasma amphiregulin levels.

In contrast, gene expression analysis revealed a molecular profile in baseline samples that showed a trend for association with response. Interestingly, a more pronounced association was observed when comparing tumor baseline profiles and skin toxicity. Generally, the results of the gene expression analysis have to be interpreted with caution because highquality tumor samples were available for less than one-third of the ITT population. There is no indication that the subjects with available samples differ in certain characteristics from the overall ITT population, but this possibility cannot be excluded. Further analyses are needed to confirm these observations and to elucidate the biological context.

In general, all experimental approaches (IHC, gene expression analysis and plasma proteomics) revealed on-treatment changes of molecular profiles. Some of these changes had a clear trend for association with response. No significant association of on-treatment changes with skin toxicity or dose escalation can be reported, but statistical analyses were often impeded by small sample sizes.

Efficacy Results (ITT Population): Subjects in the dose-escalation group B showed improved efficacy compared to subjects in the standard cetuximab group A with regard to:

- confirmed overall response rate: 29.5% (95% confidence index (CI) 16.8, 45.2) vs 15.6% (95% CI 6.5, 29.5)
- disease control rate: 70.5% (95% CI 54.8, 83.2) vs 57.8% (95% CI 42.2, 72.3)
- median duration of response (months): 6.9 (95% CI 5.6, 8.1) vs 5.8 (95% CI 5.6, 8.3)
- median PFS time (months): 4.8 (95% CI 3.8, 6.7) vs 3.9 (95% CI 2.8, 5.3) months

Median survival times were comparable: group A 10.0 (95% CI 6.0, 12.1) months, group B 8.6 (95% CI 7.3, 12.5).

Response rates for the subset of 44 subjects in group C who experienced grade >1 skin reactions during the first 3 weeks of treatment with the standard cetuximab regimen were also higher than those in group A: confirmed overall response rate 34.1% (95% CI 20.5, 49.9%), disease control rate 75.0% (95% CI 59.7, 86.8%).

PK results**:** PK parameters for the dose-escalation regimen (group B) were in good agreement with those of the weekly standard regimen (groups A and C) which agreed well with historical data. There was a linear relationship between dose and AUC_{tau} and between dose and *Cav* .

Values for half-life, clearance and volume of distribution remained at constant levels, indicating predictable PK within the dose range 250 to 500 mg/m².

Safety results (Safety population): Overall, weekly cetuximab doses of up to 500 mg/m² were well tolerated in group B; 24 (54.6%) subjects reached the highest dose. The median duration of cetuximab treatment in group B was comparable to that in group A (18.1 vs 18.0 weeks).

Dropouts**:** All subjects experienced AEs and all but 1 subject (group A) experienced treatment-related AEs. The most common AEs in all treatment groups were consistent with the underlying disease and the known side effects of cetuximab and irinotecan. Anxiety, dermatitis acneiform, dry skin, erythema, fatigue, and pruritus were all more common in group B than in group A (treatment difference ≥5 subjects). With one exception (dermatitis acneiform), the imbalance was already present before randomization and post-randomization frequencies were comparable.

Grade 3 or 4 AEs were reported in 29 (64.4%) subjects in group A, 31 (70.5%) in group B, and 57 (74.0%) in group C. There were some differences in frequencies (≥3 subjects) of individual AEs in the randomized treatment groups: general physical health deterioration, neutropenia more common in group A; diarrhea, hypomagnesemia, dry skin more common in group B. The frequency of grade 3 or 4 diarrhea in group B was comparable to that in group C. Overall frequencies of diarrhea and dry skin that started or worsened after day 22 were comparable in groups A and B. All of the cases of hypomagnesemia in group B started before randomization. The higher incidences of these grade 3 or 4 AEs in group B were not therefore due to dose escalation of cetuximab.

Skin reactions and acne-like rash were experienced by 41 (91.1%) subjects in group A and 43 (97.7%) subjects in group B. The skin reaction started during the first 3 weeks of study treatment in almost all subjects and resolved before discontinuation of cetuximab in about one-quarter of these subjects. The proportions of subjects who experienced a grade 1 skin reaction after randomization were similar: group A 17 (37.8%), group B 15 (34.1%) subjects. The proportion of subjects who experienced a ≥ grade 2 skin reaction after randomization was, however, higher in group B than in group A: 25 (56.8%) vs 16 (35.6%) subjects. Grade 3 skin reactions were experienced by 5 randomized subjects who were all in the dose-escalation group B. The severity of skin reactions in group B was thus higher than in group A. In both treatment groups the face was the most common site for skin reactions (about 90% subjects), followed by the chest and back (about 50 to 70%). Wound healing of skin biopsy sites with and without rash was similar across treatment groups; higher cetuximab doses thus had no obvious impact on wound healing.

Serious AEs were reported in 20 (44.4%) subjects in group A, 17 (38.6%) in group B, and 34 (44.2%) in group C. Most types of serious AEs occurred in only 1 or 2 subjects of one or more treatment groups. Frequencies of most serious AEs were comparable in groups A and B. Intestinal obstruction, weight decreased, renal failure were reported in 2 subjects in group B but no subjects in group A. All of these AEs apart from 1 case of renal failure were assessed as not related to study medication. In group C, 2 cases of ileus and 1 case of renal impairment were reported. All serious sepsis events (1 in group A: sepsis; 4 in group B: enterococcal sepsis 1, septic shock 1, sepsis 2) started before randomization; the imbalance was thus not due to dose escalation of cetuximab. All sepsis events were assessed as unlikely or not related to cetuximab by the investigator.

Other safety: There were no clinically relevant differences between the 2 randomized treatment groups with respect to laboratory findings, vital signs, electrocardiogram, cardiac ejection fraction, or KPS. HACAs were not detected in any of the 22 evaluated subjects.

Deaths**:** 122 (73.5%) subjects died: 37 (82.2%) in group A, 31 (70.5%) in group B, and 54 (70.1%) in group C. None of the deaths were related to cetuximab and only 1 death (group B) was related to irinotecan. 26 subjects died within 30 days after last dose of study medication: 5 in group A, 6 in group B, 15 in group C. Disease progression was the most common reason and accounted for 69.2% (18/26) of the deaths; 4 deaths were due to a disease-related complication, 2 to an intercurrent or unrelated illness or event, 1 to chemotherapy and in 1 subject the cause was unknown.

Conclusions:

- IHC analysis of skin biopsies showed that escalating the dose of cetuximab (group B) did not have any detectable effect on biomarkers associated with EGFR and the downstream signaling pathway compared to the standard cetuximab regimen (group A).

- IHC analysis of tumor biopsies showed on-treatment changes of p27, nuclear ERK and Ki67. An association with response was found for Ki67.

- Gene expression analysis of baseline tumor samples identified molecular profiles that seem to be associated with response and skin toxicity.

- Gene expression and plasma proteomic analyses revealed on-treatment changes of molecular profiles with a clear trend for association with response.

- Gene expression and plasma proteomic analyses identified individual markers and molecular profiles that have the potential to serve as prognostic or predictive biomarkers. Individual markers and profiles need confirmation and further analyses have to be performed to identify the underlying biological processes and pathways.

- Subjects in group B showed trends for improved efficacy compared to group A but treatment differences were not statistically significant: overall response rate 29.5% (13/44) vs 15.6% (7/45) subjects, disease control rate 70.5% (31/44) vs 57.8% (26/45) subjects, median duration of response 6.9 vs 5.8 months, median PFS time 4.8 vs 3.9 months. Higher response rates were also found in the subset of 44 subjects in group C who experienced grade >1 skin reactions during the first 3 weeks of treatment with the standard cetuximab regimen: objective response rate 34.1%, disease control rate 75.0%. Overall, findings were in line with those from previous studies and support the hypothesis that skin reactions are associated with improved efficacy.

- More subjects in group B than group A experienced grade ≥2 skin toxicity after randomization: 25 (56.8%) vs 16 (35.6%) subjects. There were no grade 3 or 4 skin

reactions in group A after randomization but there were 5 subjects in group B with grade 3 skin reactions after randomization. Higher cetuximab doses had no obvious impact on wound healing.

- Apart from a higher frequency of dermatitis acneiform and more severe skin reactions, the safety profile of the dose-escalation group B was largely comparable to that observed with the standard cetuximab regimen. There were no new or unexpected safety findings. Infusion of higher doses of cetuximab is safe according to the administration schedule employed.